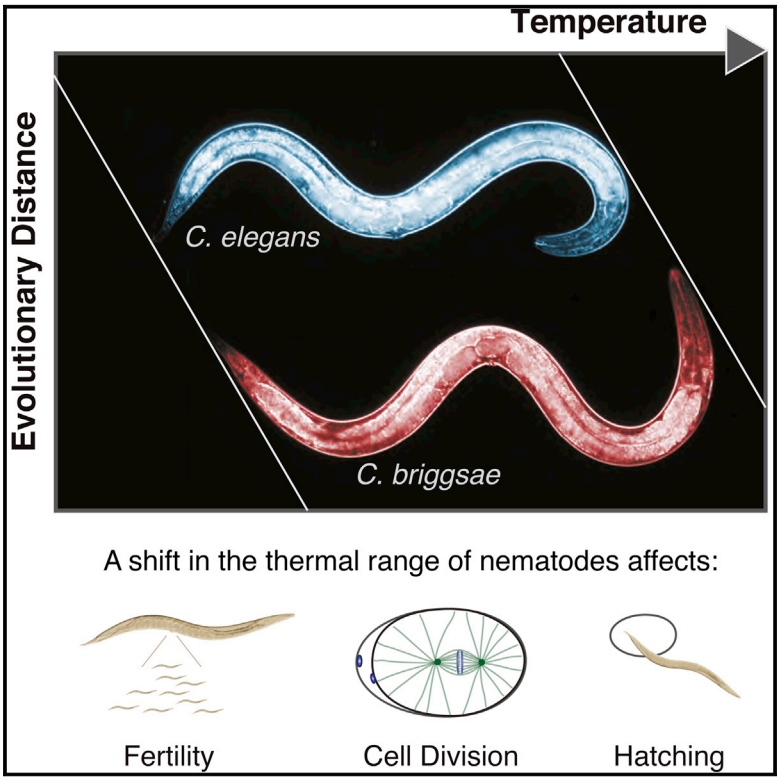
Report

Cell Reports

Temperature Dependence of Cell Division Timing Accounts for a Shift in the Thermal Limits of *C. elegans* and *C. briggsae*

Graphical Abstract



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In Brief

With climate change, it is important to understand how temperature affects the fitness of cold-blooded organisms. Begasse et al. show that the temperature dependence of cell division differs in two closely related nematodes. This shift in the temperature response has corresponding effects on development and reproductive output.

Highlights

- Timing of the first embryonic cell division follows the Arrhenius equation
- The coordination of cell division is impaired outside the Arrhenius range
- C. elegans and C. briggsae become sterile outside the Arrhenius range
- The Arrhenius range of C. briggsae is shifted to higher temperature by 2°C

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Temperature Dependence of Cell Division Timing Accounts for a Shift in the Thermal Limits of *C. elegans* and *C. briggsae*

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SUMMARY

Cold-blooded animals, which cannot directly control their body temperatures, have adapted to function within specific temperature ranges that vary between species. However, little is known about what sets the limits of the viable temperature range. Here we show that the speed of the first cell division in *C. elegans* N2 varies with temperature according to the Arrhenius equation. However, it does so only within certain limits. Outside these limits we observe alterations in the cell cycle. Interestingly, these temperature limits also correspond to the animal's fertile range. In *C. briggsae* AF16, isolated from a warmer climatic region, both the fertile range and the temperature range over which the speed of cell division follows the Arrhenius equation, are shifted toward higher temperatures. Our findings suggest that the viable range of an organism can be adapted in part to a different thermal range by adjusting the temperature tolerance of cell division.

INTRODUCTION

The body temperature of cold-blooded animals is largely determined by the ambient temperature. Therefore, they have adapted to develop and function within temperature ranges that are typical for their habitat. However, little is known about what sets the limits of the viable temperature range of species or how they adapt to a different thermal range. The early development of embryos is thought to be particularly sensitive to changes in temperature. Cold-blooded animals go to inordinate lengths to keep their developing offspring under constant temperature: bees actively regulate the temperature of their hives (Stabentheiner et al., 2010; Bujok et al., 2002; Bonoan et al., 2014), turtles return thousands of miles to the same location to lay their eggs (Bowen et al., 1993; Weber et al., 2012), and lizards take high risks to find thermally beneficial nesting sites (Angilletta et al., 2009). One of the key features of early development is rapid cell division. However, we lack understanding of how cell divisions are affected by changes in temperature. The temperature dependence of growth rates has been addressed in bacteria (Johnson and Lewin, 1946) and plants (Parent et al., 2010), but data on animals are sparse.

Caenorhabditis are ideal animals to address such questions. In their natural habitat, free-living nematodes are subject to daily and seasonal fluctuations in temperature (Félix and Braendle, 2010) and have evolved to be viable over different ranges of temperatures (Lyons et al., 1975). However, although adult worms can withstand short periods of heat shock (Lithgow et al., 1995), it affects their fertility (Aprison and Ruvinsky, 2014). Growth temperatures above 25°C are stressful for *C. elegans* and lead to sterility; this is true for natural isolates as well as the lab strain N2 (Matsuba et al., 2013; Hirsh et al., 1976; Anderson et al., 2011; Petrella, 2014). To date, five distinct clades of *C. briggsae* natural isolates have been identified. The three tested clades (Temperate, Tropical, and Nairobi) are fertile in temperatures up to 27°C or 30°C (Prasad et al., 2010; Cutter et al., 2010). *C. briggsae* AF16, isolated from India, belongs to the high-temperature-tolerant Tropical clade (Prasad et al., 2010). Despite having diverged at least 18 million years ago (Hillier et al., 2007; Cutter, 2008), *C. elegans* N2 and *C. briggsae* AF16 are almost morphologically identical with the same mode of reproduction and similar numbers of offspring. In both species, the early cell divisions are rapid, meaning that the rate of cell division is generally faster than at later stages of development (Deppe et al., 1978; Sulston and Horvitz, 1977; Sulston et al., 1983). Here, we characterize the effect of temperature on the rate of the first cell division in two nematodes adapted to different temperature niches.

RESULTS

Cell Division Time in *C. elegans* Decreases Exponentially with Temperature

We characterized the temperature dependence of the first embryonic cell division of *C. elegans* between 4.5 and 30°C (Experimental Procedures). Early one-cell embryos were shifted from 20°C to the test temperature and observed by time-lapse microscopy. The length of the interval between pronuclear meeting and cytokinesis onset (Figure 1A) is temperature dependent and

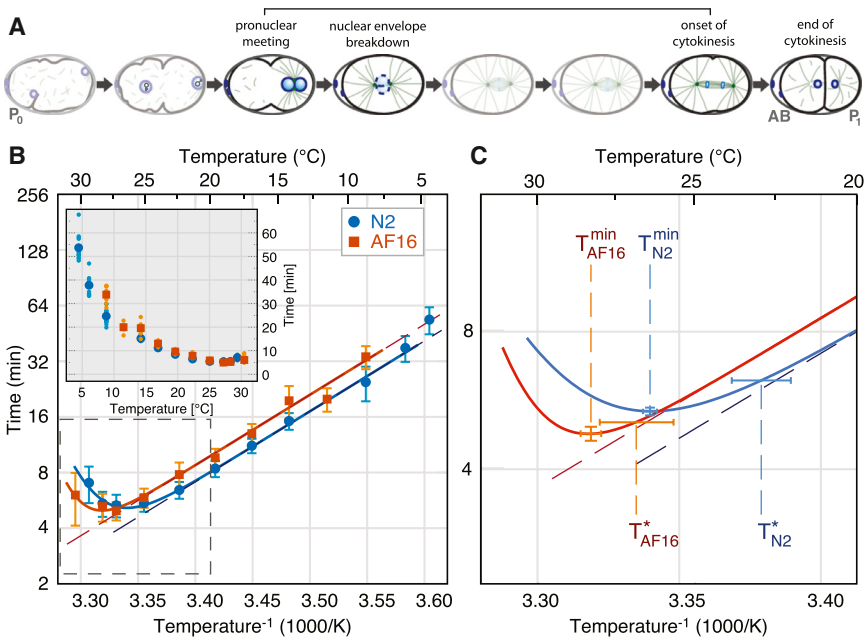

Figure 1. The Cell Cycle Interval Pronuclear Meeting to Cytokinesis Onset Is Exponentially Dependent on Temperature

(A) The events to determine cell division intervals are indicated: DNA, blue; microtubules, green. AB cell gives rise to somatic tissue. P_1 is the germline precursor cell. The anterior of the embryo is at the left; posterior is at the right.

(B) Interval length for pronuclear meeting to cytokinesis onset decreases with temperature for *C. elegans* N2 (blue) and *C. briggsae* AF16 (red). Inset: individual data points (n = 5–14) and means. Main panel: means and SDs of the original data in an Arrhenius plot: interval length on a log scale (y axis) versus inverse of temperature in kelvins (x axis). Secondary x axis: temperature in degrees Celsius. Solid line: fit with an extension of the Arrhenius equation; dashed line: Arrhenius fit.

(C) The mean fits for the bootstrapped data are shown as a magnification of the region highlighted in (B) (dashed box). T^* and T^{min} including the SD for T^*, T^{min} and $Time^{min}$ are shown. The difference in T^*, T^{min} and $Time^{min}$ between *C. elegans* and *C. briggsae* was statistically significant ($p < 0.001$). See also Supplemental Experimental Procedures, Figures S1–S4 and Table S2.

decreases from 50 min at 5°C to almost 5 min at 25°C (Figure 1B, inset, in blue). Within this temperature range, interval times fell on a straight line after logarithmic transformation of the time axis. It is interesting that this resembles the empirical Arrhenius equation, which describes the temperature-rate relationship of first-order chemical reactions. Therefore, we chose to analyze our data by fitting the Arrhenius equation

$$\mathrm{Time}_{(\mathrm{event2-event1})} = A \exp(E_a/RT)$$

(Figure 1B, main plot, dashed blue line; Experimental Procedures).

An Arrhenius fit confirms that a process is exponentially dependent on temperature if the data fall on a straight line when rates are plotted on a log scale against the inverse of absolute temperature. As shown in Figure 1B, cell division timing of the *C. elegans* embryo decreases exponentially with temperature across approximately 18°C.

The Rate of Cell Division Slows Down at High Temperature

The first embryonic cell division of *C. elegans* is sensitive to high temperature stress and fails at 30°C (Movie S1). At 28°C, cells divide, but 19.4% (7/36) of embryos failed to establish proper asymmetry in the second cell division. However, already above 25°C, cells show signs of heat stress, as cell division no longer increases exponentially as predicted by the Arrhenius equation (Figure 1B; Figure 2).

To improve the analysis of biological temperature-rate data to include data points at high temperatures, which cannot be fit by one exponential, Johnson et al. fit the growth rate of *E. coli* with a four-parameter fit based on the transition state theory of Eyring (1935), which includes a term accounting for reversible protein denaturation at high temperature (Johnson et al., 1942; Johnson and Lewin, 1946). Similarly, we developed a four-parameter equation based on the Arrhenius equation that contains an additional term to include the high temperature data:

$$\mathrm{Time}_{(\mathrm{event2-event1})} = A_1 \exp(E_1/RT) + A_2 \exp(-E_2/RT)$$

(Experimental Procedures; Supplemental Information).

With this empirical fitting method, we can characterize the temperature dependence of cell division in an unbiased way without making assumptions about what limits cell division timing at high temperatures. This also allows us to define the temperature range over which cell division follows Arrhenius kinetics by defining the characteristic temperature T^*, which is the temperature where the interval length no longer follows an exponential decrease, and the temperature T^{min}, where cell division is fastest (Figure 1C). To estimate the error associated with these parameters, the fitting was conducted on 1,000 bootstrap replicates of the data for the interval pronuclear meeting to cytokinesis onset (Figures 1C and S4; Supplemental Experimental Procedures; Table S2). The mean and SD calculated from the fits of the bootstrapped data are hereinafter given in parentheses after the values obtained from the original data set.

The interval between pronuclear meeting and cytokinesis onset decreased exponentially with increasing temperature up to a T^* of 23.6°C (23.0 ± 0.9), followed by a nonexponential decrease to a minimum interval length at T^{min} 26.4°C (26.4 ± 0.2), after which the interval length increases between 27 and 29°C (Figures 1B and 1C, in blue). It is interesting that *C. elegans* becomes sterile beyond the temperature at which the embryo reaches the maximum rate of cell division, 26.4°C (Figure 3B). This finding emphasizes that deviation from Arrhenius, which ultimately leads to a slowing down of cell division,

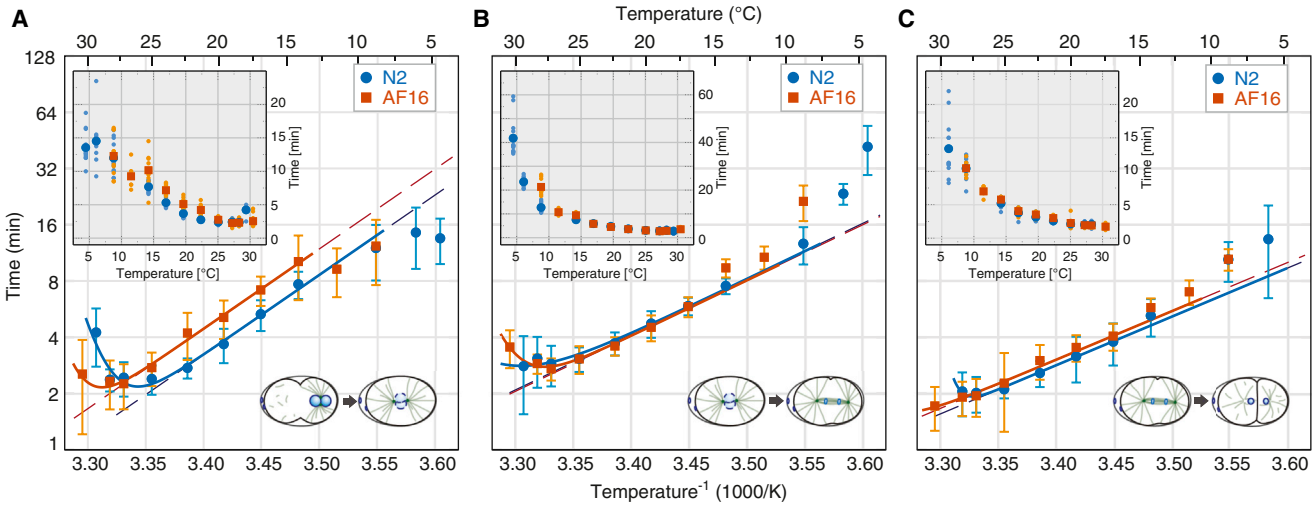

Figure 2. Temperature Dependence of Three Consecutive Intervals Follows Different Slopes

(A) The interval from pronuclear meeting to nuclear envelope breakdown.

(B) The interval from nuclear envelope breakdown to cytokinesis onset.

(C) The interval from the cytokinesis onset to the end of cytokinesis.

Insets: individual data points (n = 5–14) and means. Main panels: means and SDs of the original data in an Arrhenius plot (axes are as for Figure 1). *C. elegans* N2 is in blue; *C. briggsae* AF16 is in red. Solid line: fit with an extension of the Arrhenius equation; dashed line: Arrhenius fit.

See also Supplemental Experimental Procedures, Figures S2 and S3, and Table S2.

is an indicator of cellular stress and affects the development of the organism.

The Reproductive Temperature Range Is Shifted to Higher Temperatures in *C. briggsae*

We next asked if the correlation between the Arrhenius range of cell division and the fertile range holds true in nematodes that survive higher temperatures. Variation in fitness between *C. elegans* isolates at 25°C has previously been reported (Harvey and Viney, 2007), but none of the 12 natural isolates of *C. elegans* we tested (Table S1) were fertile at 27°C. Therefore, we compared the survival of another well characterized *Caenorhabditis* strain: *C. briggsae* AF16, which was reported to be fertile up to 30°C (Prasad et al., 2010). A single *C. briggsae* AF16 hermaphrodite could form a population after a 5°C increase in temperature from 25 to 30°C, but *C. elegans* N2 could not (Figure 3A). This experiment for *C. elegans* simulates a local extinction of a nematode not adapted to survive at high temperatures (Félix and Duveau, 2012). To assess the fitness across their full fertile temperature range, we compared the lifetime fecundity of *C. elegans* and *C. briggsae* hermaphrodites by moving individual eggs to the test temperature and counting their progeny (Experimental Procedures). In our hands, both species were viable for several generations over a span of 15°C: *C. elegans* between 10 and 25°C and *C. briggsae* between 14 and 29°C. *C. elegans* becomes sterile in the second generation at 27°C, and *C. briggsae* does so at 30°C. Lifetime fecundity is highly temperature dependent, with low numbers of offspring at cold and hot temperatures and over 300 offspring at the optimal temperature. The temperature at which single *C. elegans* and *C. briggsae* hermaphrodites gave rise to the highest number of offspring was determined by fitting a parabola to the data (Figure 3B). The optimal temperature was significantly shifted between both species: 18.2°C for *C. elegans* and 21.5°C for *C. briggsae*. Considering the shift in the fertile range and the optimal temperatures, we estimated a shift of 3–4°C in reproductive fitness between *C. elegans* and *C. briggsae*.

The Temperature Dependence of Cell Division Is Shifted to Higher Temperatures in *C. briggsae*

These observations prompted us to ask how the cell cycle length of *C. briggsae* responds to different temperatures. If there were a link between the fertile range and the temperature dependence of cell division, then we would expect the rate of *C. briggsae* cell division to continue to increase exponentially up to a higher temperature; alternatively, the temperature response should be the same as it is for *C. elegans*. Analysis of *C. briggsae* one-cell embryos revealed that the interval from pronuclear meeting to cytokinesis onset again showed an exponential decrease over a range of 18°C. However, the exponential range extends toward warmer temperatures. The breakpoint temperature T* of *C. briggsae* is at 26.6°C (26.9 ± 1.2; Figures 1B and 1C), 3.0°C higher than in *C. elegans*. The minimal interval length T^{min} is at 28.3°C (28.3 ± 0.3), 1.9°C higher than in *C. elegans* (Figure 1C, in red; Table S2). The minimal interval length was similar in *C. elegans*, 312 s (320 ± 6), and *C. briggsae*, 300 s (294 ± 10). It is striking that data from both species could be fit with the same slope across the Arrhenius range (Figure 1B; Table S2; Supplemental Experimental Procedures). At each temperature in this 9–25°C range, cell division was slower in *C. briggsae* compared to that of *C. elegans*. Therefore, we tested if the *C. briggsae* data could be shifted along the temperature or time axis to match the *C. elegans* data. Indeed, a

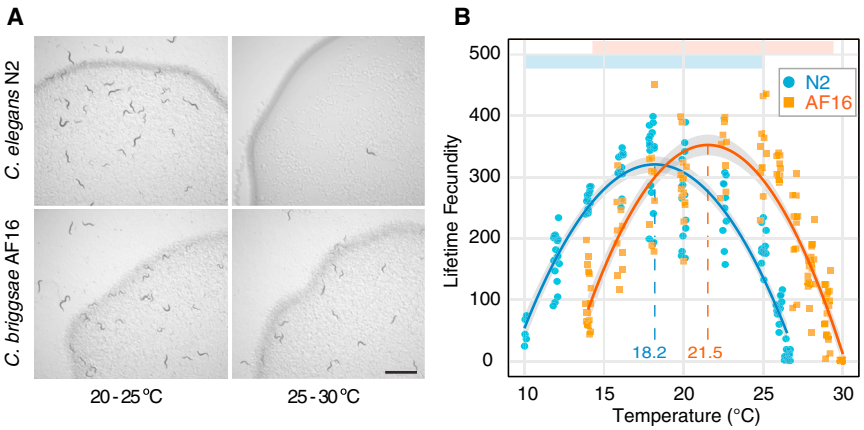

Figure 3. The Fertile Range of *C. briggsae* AF16 Is Shifted to a Higher Temperature

(A) Single hermaphrodites of *C. elegans* N2 (top) or *C. briggsae* AF16 (bottom) were raised from eggs at 20 or 25°C and shifted up by 5°C as adults. Three days later, the plates were scored for the ability to form a population. Scale bar, 2 mm.

(B) Total lifetime fecundity of individual hermaphrodites (n = 9–18) of *C. elegans* N2 (blue) and *C. briggsae* AF16 (orange). Data were fit by a second-order polynomial. Optimal temperatures are indicated. Gray shading: the 95% confidence region of the respective fit. Comparison of the parabolic models by ANOVA confirmed a significant shift between the two species ($p < 0.001$). Colored shading on the top: temperature ranges where the worms were fertile for several generations.

shift by 1.9°C toward lower temperatures resulted in a good overlay (Figure S1). Thus, the Arrhenius range of *C. briggsae* cell division is shifted by about 2°C toward higher temperatures, compared to that of *C. elegans*. This confirms the connection between the fertile range and the temperature dependence of cell division.

Different Intervals of the Cell Cycle Show a Different Response to Temperature that Is Conserved between *C. elegans* and *C. briggsae*

To test if different aspects of cell division are affected to the same extent by temperature or if one process is rate limiting at temperature extremes, we analyzed shorter intervals in both species with our modified Arrhenius equation (Figure 2). Fitting of the intervals from pronuclear meeting to nuclear envelope breakdown and from nuclear envelope breakdown to cytokinesis onset (Figures 2A and 2B; these two intervals together make up the interval pronuclear meeting to cytokinesis onset, which we have already presented), as well as from cytokinesis onset to end of cytokinesis (Figure 2C), revealed that the timing of all analyzed aspects of cell division change with temperature. However, not all intervals of the same species (*C. elegans* or *C. briggsae*) could be fit with the same slope (Figure 2; Figure S2). In chemistry, this would indicate that the timing of the intervals is determined by different reactions. Analysis of these intervals confirmed our previous observations in two ways: first, the Arrhenius range for the same intervals of *C. elegans* and *C. briggsae* could be fit with the same slope; and second, the breakpoint temperature T* is always at a higher temperature by 2–3°C in *C. briggsae* than in *C. elegans* (Figure 2; Table S2).

It also revealed that not all rates follow Arrhenius at cold temperatures. In *C. elegans*, the interval from pronuclear meeting to nuclear envelope breakdown is shorter than predicted for an exponential increase at temperatures below 10°C (Figure 2A, blue dotted line). However, for the interval from nuclear envelope breakdown to cytokinesis onset, the cell cycle interval is longer than expected (Figure 2B, blue dotted line). In fact, it seems that the timing of nuclear envelope breakdown is sensitive to cold temperatures and occurs earlier than predicted by Arrhenius kinetics when cells from *C. elegans* are placed below 10°C and cells from *C. briggsae* are placed below 14°C. Taking all analyzed intervals into account, this results in a 15°C range, where all aspects of cell division timing follow Arrhenius. Intriguingly, the temperatures at which tight coordination of different aspects of cell division is lost correspond to the lower limits of fertility in both species.

The Upper Temperature of Successful Embryonic Development Is Shifted between *C. elegans* and *C. briggsae*

We next asked if the observed shift in the Arrhenius range of cell division between *C. elegans* and the high-temperature-tolerant *C. briggsae* is relevant for the development of the organism. We monitored the rate of hatching success from one-cell, two-cell, or four-cell embryos to determine the thermal sensitivity of embryonic development (Figure 4; Experimental Procedures). In general, the hatching success of one-cell embryos was lower than for later embryos. We speculate that they are more sensitive to manipulation as the eggshell in early one-cell embryos is not yet fully formed (Olson et al., 2012). Hatching success declined below 80% for one-cell embryos at 28°C for *C. elegans* and 30°C for *C. briggsae*. Embryonic development fails at 30°C for *C. elegans* and at 32°C for *C. briggsae* (Figure 4B). These data show that the upper temperature limit of successful embryonic development is shifted by 2°C between *C. elegans* and *C. briggsae*—the same temperature shift we found for the Arrhenius range of cell division.

DISCUSSION

Our most important finding is that the first cell divisions of *C. elegans* and *C. briggsae* embryos show the same kinetics as first-order chemical reactions across their fertile temperature range: they follow the Arrhenius equation. The rates of many other complex biological processes also have Arrhenius-like kinetics (Crozier and Stier, 1926; Pütter, 1914; Vanoni et al., 1984), including the flashing of fireflies and the speed of ants (Laidler, 1972). In chemistry, the rate-limiting reaction can often be deduced from the Arrhenius fit. In complex biological systems, such an approach is not feasible. Nevertheless, the obtained parameters can be used to compare the temperature dependence of cell division between the two species. By making

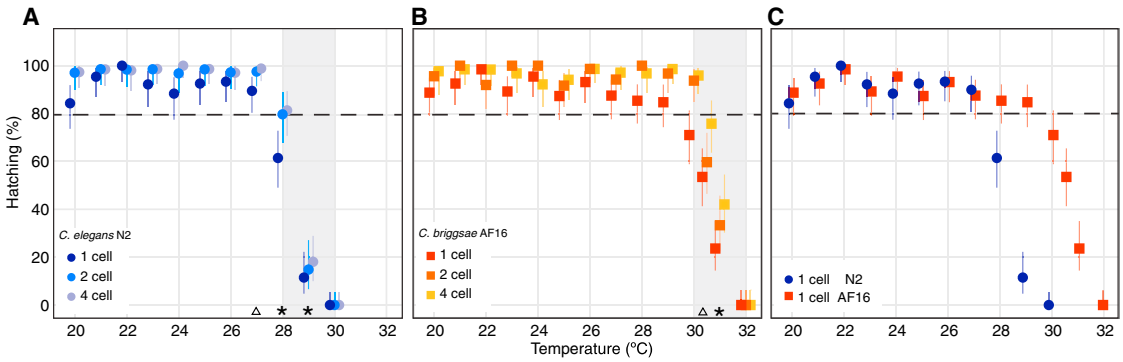

Figure 4. Embryonic Development Fails at High Temperatures

(A and B) The hatching frequency of one-, two-, and four-cell embryos at different temperatures of (A) *C. elegans* N2 and (B) *C. briggsae* AF16.

(C) Comparison of the hatching frequency from one-cell stage embryos of *C. elegans* N2 (blue circles) and *C. briggsae* AF16 (red squares).

Below 80% of hatching success (dashed line) marks the temperature range of declining hatching frequency (gray shading). Means of 43–75 embryos with binomial confidence intervals are staggered at each temperature to improve readability. Symbols: △, temperature where hatched larvae become sterile adults; *, worms die as larvae.

the analogy to chemical reactions, we can speculate on how the timing of cell division is regulated. Since different intervals of the *C. elegans* and *C. briggsae* cell cycle could be fit by the same slope across the Arrhenius range, it suggests that cell cycle control might be constrained and is conserved as an organism adapts to different temperatures.

This study aimed to investigate what sets the limits of the viable temperature range in nematodes. We found that cell division fails to follow Arrhenius kinetics outside the fertile temperature range of *C. elegans* and *C. briggsae.* Similar results have been obtained for the temperature dependence of larval development (M. Olmedo, M. Geibel, and M. Merrow, personal communication). Larval growth speed increases with temperatures but slows down again beyond 25.5°C. This leads us to speculate that biological systems can function over the range of temperatures where physiological rates follow Arrhenius-like kinetics. Such behavior might naturally allow distinct cell biological processes to remain coordinated. For instance, we found that once cell cycle timing deviates from the exponential temperature dependence, coordination between different aspects of the asymmetric first cell division is no longer ensured, and development is impaired. Our analysis suggests that biological systems have evolved to stay synchronized as long as rates are exponentially temperature dependent.

The 2–4°C shift between *C. elegans* and *C. briggsae* in the Arrhenius range of cell division, embryonic development, and fecundity indicates that adaptation to a different temperature range is possible. It is interesting that the gain in fitness of *C. briggsae* at high temperatures comes at a loss of fitness at low temperatures. It is likely that selection favored the ability of *C. elegans* N2 (from England) to withstand cold temperatures. Since we observed a shift, and not an extension, of the Arrhenius and fertile ranges, we speculate that adaptation may not require multiple independent mutations that affect only one tissue or cell type; rather, that the thermal sensitivity of the whole organism can be shifted in response to evolutionary pressure. This notion is supported by Kuntz and Eisen's (2014) study on *Drosophila* isolated from different climates. They found that developmental timing varied greatly between species if compared at one temperature but that the different developmental stages of all species scaled uniformly with temperature. A captivating idea to be tested is that the availability of energy in the form of ATP is the unifying mechanism that drives all cellular rates and governs the temperature dependence of cells and tissues. In this case, the maximum rate of mitochondrial activity might limit the rate of cell division. Experimental manipulation of cellular respiration by RNAi slows down embryonic cell cycle rates and the overall progress of development (Sönnichsen et al., 2005) (data not shown). Mitochondrial properties are also an attractive candidate to explain the different upper temperature limits between *C. briggsae* natural isolates. If this difference was encoded in the mitochondrial DNA, this hypothesis could be tested by integrating the mitochondrial genomes of high-temperature-tolerant *C. briggsae* into the genetic background of temperature-sensitive isolates. Natural isolates from the high-temperature-tolerant Tropical clade have comparably lower mitochondrial membrane potential and lower levels of reactive oxygen species (Hicks et al., 2012). This fits with our observation that *C. briggsae* AF16 has slower cell cycle rates and an increased resistance to stressful temperatures. Also, the observed shift in T^* and T^{min} could be analogous to an oxygen-concentration-dependent shift of the breakpoint temperatures in Arrhenius plots of the respiratory rates of leeches and frogs (less oxygen at high temperature could limit ATP production via the electron transport chain) (Crozier, 1926; Crozier and Stier, 1926; Pütter, 1914).

Our data show that, in *C. briggsae* AF16, embryonic development and fecundity start to fail at the same temperature, 30°C. This is not the case in *C. elegans* N2, where embryonic development declines at 28°C but worms become sterile after 25°C. This indicates that an aspect of germline development or fertilization in *C. elegans* is highly temperature sensitive but more robust in *C. briggsae* AF16. It is known that thermal tolerance is increased in conditions of enhanced stress resistance (Lithgow et al., 1995). Therefore, an additional heat shock response could explain the resistance of *C. briggsae* AF16 to high temperatures. A lack of such a heat shock response might be the reason why no

C. elegans isolate has been found that can be cultivated beyond 25°C (Anderson et al., 2011; Hirsh et al., 1976) (Table S1). Alternatively, it is also possible that *C. elegans* has experienced less selective pressure during evolution to adapt to high temperature, perhaps because temperatures above 26°C induce the resistant dauer stage that can survive harsh conditions (Ailion and Thomas, 2000). Dauer formation is not induced in *C. briggsae* AF16 at high temperatures (Inoue et al., 2007).

The fact that a 2°C shift in temperature can be so deleterious to early cell divisions suggests why ectothermic and egg-laying species are vulnerable to climate change. Our data show that there are likely to be adaptive mechanisms to ensure successful cell division at varying temperatures, at least in the diverse phylum of nematodes. So far, we have only looked at two different species, so we cannot say whether this indeed represents an adaptive mechanism. Further analysis in different species and a molecular understanding of these mechanisms will be essential steps in assessing how rapidly organisms can adapt to changing temperature.

EXPERIMENTAL PROCEDURES

Worm Handling

C. elegans N2 and *C. briggsae* AF16 were maintained as described elsewhere (Brenner, 1974). The temperature 20°C was chosen for cultivation, as it is the temperature with which both species seemed equally fit, based on fertility. Strains were supplied by the Caenorhabditis Genetics Center (University of Minnesota), which is funded by the NIH Office of Research Infrastructure Programs (P40 OD010440).

Temperature-Controlled Imaging

One-cell embryos were dissected from hermaphrodites in M9 buffer at 20°C and mounted on agarose pads. Temperature-controlled differential interference contrast (DIC) microscopy was achieved using a custom-made jacket, fitting the oil-immersion objective, which was temperature regulated by a water bath. Room temperature was at 20°C for imaging at 15°C and higher and at 4°C for experiments below 15°C. The 12°C data point for *C. elegans* is omitted, as we could not keep this temperature stable after a change of seasons. Images were acquired every 6–20 s, depending on acquisition temperature. Our data were obtained after shifting the early embryo from 20°C to the test temperature. Cellular rates responded to the shift in temperature within seconds. Alternatively, one could have cultivated worms across a temperature range to let them adjust to the test temperatures. However, this approach would not have allowed us to gather data for the stressful temperatures, at which worms become sterile.

Analysis of Cell Cycle Intervals

The events to determine cell division intervals were tracked manually from DIC time-lapse images in ImageJ (http://rsbweb.nih.gov/ij/index.html). The exponential range of the data was fit by the Arrhenius equation, using time instead of rates: $\mathrm{Time}_{(\mathrm{event2}\ -\ \mathrm{event1})} = A\ \exp(E_a/RT)$, where time (in minutes) is the interval between two events, E_a (in kilojoules per mole) is the activation energy, A (in minutes) is the frequency factor, R (in joules per mole kelvin) is the gas constant, and T is the temperature in kelvins. To include the high temperature points, a two-exponential expression was used:

$$\mathrm{Time}_{(\mathrm{event2-event1})} = A_1 \exp(E_1/RT) + A_2 \exp(-E_2/RT);\ E_1>0,\ E_2>0$$

(Supplemental Experimental Procedures; Table S2).

T* was defined by a 5% deviation from the Arrhenius fit. T^{min} is the temperature at which the shortest interval time is reached. Only movies in which cell division was completed successfully were analyzed. The mean and SD of T*, T^{min}, and $Time^{min}$ were estimated from fits after bootstrapping with 1,000 random resamples (Supplemental Experimental Procedures; Figure S4; Table S2). These parameters were then compared between *C. elegans* and *C. briggsae* by t test using the estimated mean and SD. For all tests, the significance level (type 1 error) was set to $\alpha = 0.001$.

Lifetime Fecundity

The total number of offspring was counted for individual hermaphrodites. Hermaphrodites were raised at the test temperature from eggs laid at 20°C and moved to new prewarmed OP50 plates at regular intervals to avoid overcrowding. Worms that did not survive until egg laying ceased were excluded from analysis. Their progeny developed at the test temperature and were counted at the L4 larval stage. The optimal temperature for fecundity was determined by fitting a parabola to fecundity versus temperature data for *C. elegans* and *C. briggsae* separately. These parabolic models were then compared against each other in an ANOVA using R. For all tests, the significance level (type 1 error) was set to $\alpha = 0.001$.

Hatching Success

Embryos were dissected in M9 buffer from adults at 20°C and mouth pipetted at the one-cell, two-cell, or four-cell stage onto prewarmed *E. coli* OP50 spotted agar plates at the test temperature. Plates were kept at the test temperature until dead eggs and hatched larvae were counted. Worms were tested for larval arrest and fertility for two generations. Binomial confidence intervals were calculated with Binconf, Exact Method in R.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, four figures, two tables, and one movie and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2015.01.006.

AUTHOR CONTRIBUTIONS

M.L.B., M.L., S.W.G., and A.A.H. designed the study; M.L.B. performed the experiments with support from M.L.; M.L.B. and F.V. analyzed the data; F.V. developed the fitting of the temperature dependence of cell division; M.L.B., M.L., and A.A.H. wrote the manuscript, with support from F.V. and S.W.G.

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